Liquid semen quality of PO cattle suplemented red fruit oil (RFO) in ringer lactate-egg yolk equlibration temperature

Nurcholis, S M Salamony, and D Muchlis

Department of animal husbandry, Musamus University. Merauke, ID.

nurcholis@unmus.ac.id

Abstract. The use of Papuan red fruit as a supplement to the extender is one solution to the high price of raw material extenders. This study was aimed to analyze the effect of the use diluents supplemented with red fruit oil (RFO) in various levels against the quality of PO cattle semen during the storage of equilibration temperature. The research was conducted in the Animal Health Laboratory of Semangga District. The method used was a factorial completely randomized design (F-CRD), 3 treatments x 3 replications and 3 factors of equilibration temperature, namely 2^{o}C, 4^{o}C and 6^{o}C. This study used 4 diluent treatments, ie P0 = ringer lactate egg yolk (RL-KT), P1 = (RL-KT + 0.1 ml MBM), P2 = (RL-KT + 0.3 ml MBM). Semen is collected using an artificial vagina, shortly after the semen storage is evaluated macroscopically and microscopically. The results of the study have a significant effect (P <0.05) in treatment P0 and P1,P2 during the 48-hour equilibration time at 6^{o}C. In conclusion, supplementation of red fruit oil (RFO) in diluents was able to maintain motility up to 68,5±1.02% at equilibration temperature.

1. Introduction

Merauke is the eastern region of Indonesia which is used as a center for strengthening the production of local cattle seeds including PO cattle. [8] stated that cattle capable of developing in Merauke are types of cattle that are tolerant to climate and feed in this region. Improving the genetic quality of local cattle is needed to increase production, one of them is by utilizing artificial insemination technology and the production of raw materials for frozen semen from males. The process for producing frozen semen is to consider the quality of the liquid semen produced by the male. Some stages before producing frozen semen is the process of evaluating semen, diluting and equilibrating liquid semen.

The semen dilution process is generally needed to protect sepermatozoa from cold shock, so that the diluent raw material must be able to protect it, including materials that have antioxidants, phospholipids, omega-3 and Polyunsaturated faty acid (PUFA), besides diluents agents must be cheap and easy obtained. In general, easy and inexpensive diluents and are often used are ringer lactate and egg yolk. Egg yolks contain low density lipoproeins (LDL) that are associated with spermatozoa membranes. [4] states that the use of egg yolk in the equilibration process is able to maintain spermatozoa in cold stress and prevent the loss of phospholipids from cell membranes. In addition, in

reaping red fruit oil is produced and contains antioxidants and omega-3 which is useful for the dilution process. The main content of antioxidant red fruit oil is vitamin E and ofer-tocopherol, omega-3 and unsaturated fatty acids that are useful for cell membranes [1].

Equilibration is the time for the gradual process of temperature drop on spermatozoa which functions to prevent cold shock. The equlibration process in diluted semen requires time for the adaptation of spermatozoa at 5ºC 2-6 hours. [2] the equilibration process is carried out at 4-5ºC for 24 hours to balance intracellular fluid spermatozoa before freezing semen[2]. Other studies of the equilibration process were carried out for up to 72 hours [3] and 96 hours [6]. The use of diluents in the equilibration process finally determines the success of semen, therefore this study aims to see the ability of spermatozoa diluted with ringer lactate and egg yolk which is prepared by red fruit oil at different equilibration temperatures for up to 48 hours.

2. Methodology

The study was conducted at the semangga district animal health laboratory. Fresh semen was collected using an artificial vagina in the morning from 12 PO cattle on an average age of 3-4 years fed fresh grass. The fresh semen used in this study was semen with a mass of ++ and individual motility of at least 70%. Fresh semen is evaluated macroscopically and microscopically, quantified semen is evaluated microscopically, motility, viability and abnormality.

2.1. Data collection techniques

Comparisons between spermatozoa and 1: 9 diluents were stored at 2ºC, 4ºC and 6ºC for 48 hours. The diluent consists of P0 (7.5 ml RL + 2.5 ml EY), P1 (7.5 ml RL + EY 2.49 ml + 0.1 ml RFO), P2 (RL 7.5 ml + EY 2.47 ml + RFO 0.3 ml). Fresh semen collected from the collection was analyzed macroscopically including volume, pH and consistency, and microscopic includes motility, viability and abnormalities of spermatozoa. Motility observations were carried out with an enlargement of 10 x 10, observations of viability and abnormality were carried out by making preparations using eosin negrosine, and observing 10 x 40 magnification. Observation of viability and abnormalities was done by looking at a minimum of 200 spermatozoa cells in 10 observations in one preparation.

2.2. Data analysis techniques

The preliminary data analysis was conducted to find out the data was normally distributed, and general linear model (GLM) analysis was carried out, the next experiment comparing the diluents and the equilibration time was performed statistically using the chi-square procedure by looking at the average standard error and the significant effect $P < 0.05$ with SPSS version 21.0 software.

3. Results and Discussion

The equilibration process in this study uses fresh semen which has an average value of semen quality in macroscopic and microscopic terms such as table 1.

3.1. Quality fresh semen PO Cattle

The results of evaluation of fresh semen in table 1 show that the average individual motility is 78.33 ± 2.71% which is the semen requirement can be diluted for the equilibration process. [1] states that the volume of fresh semen of cattle is 4 mL - 4.5 mL on average, but other findings indicate that the volume of cattle semen is 5.9 ± 1.9 mL [10] and normal volume according to [3] which is between 5-8 ml. Volume differences that occur can be caused by differences in the type of livestock, age, feed given and libido level.

Table 1. Quality of fresh semen of PO cattle (n = 12)

Characteristics	Total
Macroscopic	
Volume (mL)	3,7 ±1.43
pH	7±0
Consistency	medium - thick
Microscopic	++
Mass Motility	78,33±2.71
Motility (%)	83,75±5.23
Viability (%)	2.104±105.34
Concentration (10^6/mL)	5.2 ± 2.27
Abnormality (%)	

3.2. Effect of diluents on quality of equlibration semen

The equilibration process of fresh semen with diluent supplemented with red fruit oil at a temperature of 2oC, 4oC, 6oC with a duration of 48 hours, showed a significant level of motility table 2.

The use of P2 diluents was able to maintain the motility of spermatozoa during the equilibration process up to 68.5 ± 1.02% for 48 hours and 10% decrease in fresh semen. The equilibration temperature of 6^{o}C was able to maintain a decrease in motility of 13% in the control treatment, diluents P1 and P2 had a significant effect (P <0.05) on motility at 48 hours of storage temperature of 6^{o}C. This difference in motility is affected by red fruit oil supplemented in diluentss, red fruit oil contains PUFA, omega-3 which is able to maintain permebiality and prevent expansion of phospholipids in spermatozoa cell membranes. [7] that protection by phospholipids on spermatozoa cells occurs in the head and tail. Red fruit oil (RFO) supplemented in diluents is able to maintain the viability of spermatozoa at 48 hours of treatment. Viability of spermatozoa is influenced by the state of the head spermatozoa cell membrane, [6] damaged cell membranes can cause weakening of viability. Spermatozoa that live after being given eosin show that the plasma membrane is intact, so that sodium expenditure can function properly. The Na + K + ATP-ase enzyme found in the cell's plasma membrane will pump back the Na + ion which binds to the eosin dye out of the cell. This happens naturally because the concentration of Na + ions in the cell is much lower than outside the cell.

Table 2. Quality of motility, viability spermatozoa equilibrated for 48 hours

Parameter	Diluents	Equilibration time (h) mean ± SE		
		2°C	4°C	6°C
Motility (%)	P0	68.0±2.32^{aA}	66.0±1.^{67aA}	55.5±1.03^{bA}
	P1	72.5±1.64^{aB}	68.0±1.08^{bB}	65.0±0.39^{cB}
	P2	74.5±1.11^{aB}	70.5±0.15^{bB}	68.5±1.02^{cC}
Viability (%)	P0	70.5±2.12^{aA}	65.5±1.42^{aA}	50.5±1.52^{abA}
	P1	75.5±0.11^{aAB}	70.5±3.16^{aAB}	55.0±1.52^{abAB}
	P2	75.0±1.33^{aAB}	72.0±1.00^{aB}	60.5±1.52^{abAB}

Different lowercase letters on the same line are significant (P <0.05), different uppercase letters in the same column (P <0.05). P0 (RL 7.5ml + EY 2.5ml), P1 (RL 7.5 ml + EY 2.49 ml + RFO 0.1 ml), P2 (RL 7.5 ml + EY 2.47 ml + RFO 0 + RFO 0) , 3 ml)

4. Conclusions

The equilibration process of fresh semen of PO at temperature of 2°C, 4°C and 5°C supplemented with 0.3 ml red fruit oil (RFO) was able to maintain spermatozoa motility up to 68.5 ± 1.02% and viability 60.5 ± 1.52% for 48 hours.

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